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Using phage lytic enzymes to kill pathogenic bacteria

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13. ABSTRACT (Maximum 200 words)

Bacteriophage lytic enzymes are highly evolved molecules used by the phage to quickly destroy the bacterial cell wall to release bacteriophage progeny. We have exploited the rapid and lethal action of these enzymes to destroy pathogenic bacteria. These enzymes are specific for the species or strain from which they were produced, thus avoiding destruction of the surrounding normal commensal organisms found on mucosal surfaces. We now have enzymes that are specific for *S. pyogenes, S. pneumoniae,* and *B. anthracis* with enzyme for *S. aureus, E. faecalis/E. faecium* and group B streptococci in progress. Our results using any of the phage lytic enzymes show that in vitro, 10^7 bacteria can be sterilized seconds after enzyme contact. In animal model experiments, we were able to colonize mice with either streptococcal or pneumococcal species (orally or nasally) and remove them completely with phage enzymes delivered to these sites. In a septicemia model using *S. pneumoniae,* bacteria are reduced by >2-logs from the blood of infected animals with a single intravenous dose of enzyme. In subsequent experiments we plan to use constant infusion to clear organisms from blood. Thus, phage lytic enzymes are a new reagent that may be used to control pathogenic bacteria.

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FINAL REPORT

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Project Goals: We have developed a novel method to kill pathogenic bacteria safely and quickly. This method exploits the rapid yet specific activity of bacteriophage lytic enzymes to destroy pathogenic bacteria on contact. During the course of this grant we planned to develop enzymes that control *S. pyogenes, S. pneumoniae, S. aureus, E. faecalis* and group B streptococci. In preliminary experiments in vitro, 10 nanograms of the *S. pyogenes* phage enzyme could kill 10^7 streptococci in five seconds. In vivo, we were able to remove colonizing streptococci from the nasopharynx of heavily colonized mice. Our goal was to identify enzymes from a variety of phage active on several pathogens and used these enzymes to destroy these bacteria in blood of infected animals.

Final Report: During the tenure of this grant we were successful in identifying phage enzymes for a variety of pathogens. We now have developed enzymes that are specific for *S. pyogenes, S. pneumoniae,S. aureus, E. faecalis/E. faecium* and group B streptococci. Our results show that in vitro 10^7 bacteria can be reduced to sterility seconds after enzyme contact. In animal model experiments, we were able to colonize mice with either streptococcal or pneumococcal species (orally or nasally) and remove them completely with phage enzymes delivered to these sites using a single enzyme dose. In a septicemia model with *S. pneumoniae,* bacteria are reduced by >2-logs from the blood of infected animals with a single intravenous dose of enzyme. For the group B streptococcal enzyme we developed a vaginal model of colonization and found that a single dose of enzyme will remove colonizing group B strep two hours after treatment.

We found that two enzymes with different cell wall specificities work synergistically resulting is more efficient killing ability. In addition, when we searched for the presence of bacteria resistant to their respective enzymes, none were found in the enzymes we isolated, indicating that resistance is a rare event, rarer that antibiotic resistance.

Stability studies revealed that in general, these enzymes are quite stable and are able to withstand temperatures as high as 45C for several hours and may be lyophilized for extended periods of time (18 months for the pneumococcal enzyme) and be fully active after reconstitution.

Pharmacokenetic experiments with the pneumococcal enzyme revealed that the half-life of this enzyme in blood is about 20 minutes, thus for this application multiple doses need to be administered or a constant i.v. infusion must be performed to eliminate all bacteria.

Thus, phage lytic enzymes are a new reagent that may be used in hospitals, nursing homes and the general population to control antibiotic resistant pathogenic bacteria in blood and on mucosal surfaces, offering a capability previously unavailable.

Published papers:

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